Abstract

Characterization of Cas9-mediated genome editing in human T cells

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Introduction

Genome editing via CRISPR/Cas technology promises to provide a novel class of therapies for a variety of human diseases. To unlock the potential of the CRISPR/Cas9 technology, a deeper understanding of the efficacy in different primary cell types is required. A cell type of particular interest for gene editing is the human T cell due to its central role in the evolving cancer immunotherapy landscape.

Methods

Comparison of Cas9-mediated editing by RNP and mRNA in Jurkat T cells

A) Jurkat T cells were electroporated with either S. aureus Cas9 mRNA and PDCD1 gRNA (P-293s) or S. pyogenes Cas9 (P-293s) targeting PDCD1. Cells were re-plated in RPMI1640 and counted for 3 consecutive days following staining with trypan blue.

B) The electroporated cells were collected at day 2 and 3. Point electroporation was monitored with an APC-CD3 antibody and analyzed by FACS. Quantification of the CD3-negative population is shown. C) NHEJ results from the T7E1 assay performed on the TRAC locus.

C) Quantification of the CD3 negative population from the plots in (C). E) % CD3 expression

Successful editing of naive T cells by RNP delivery

A) S. aureus Cas9 and TRAC gRNAs were transfected with P-293s. Cells were re-plated in RPMI1640 and counted for 3 consecutive days following staining with trypan blue. B) The electroporated cells were collected at day 2 and 3. Point electroporation was monitored with an APC-CD3 antibody and analyzed by FACS. Quantification of the CD3-negative population is shown. C) NHEJ results from the T7E1 assay performed on the TRAC locus.

Summary

Cas9-mediated T cell editing using Cas9 from both S. aureus and S. pyogenes.

Delivery of Cas9 as an RNP yields better viability and cutting efficiency for the PDCD1 gRNA, PDCD1-108.

Nearly 45% gene editing in Jurkat T cells at the PDCD1 locus

Generation of nearly 25% CD3 Jurkat T cells using RNP delivery

Generation of approximately 18% CD3 T cells when targeting the TRBC locus in activated T CD4 T cells

Over 15% gene editing in naive T cells was achieved using RNP against TRAC

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